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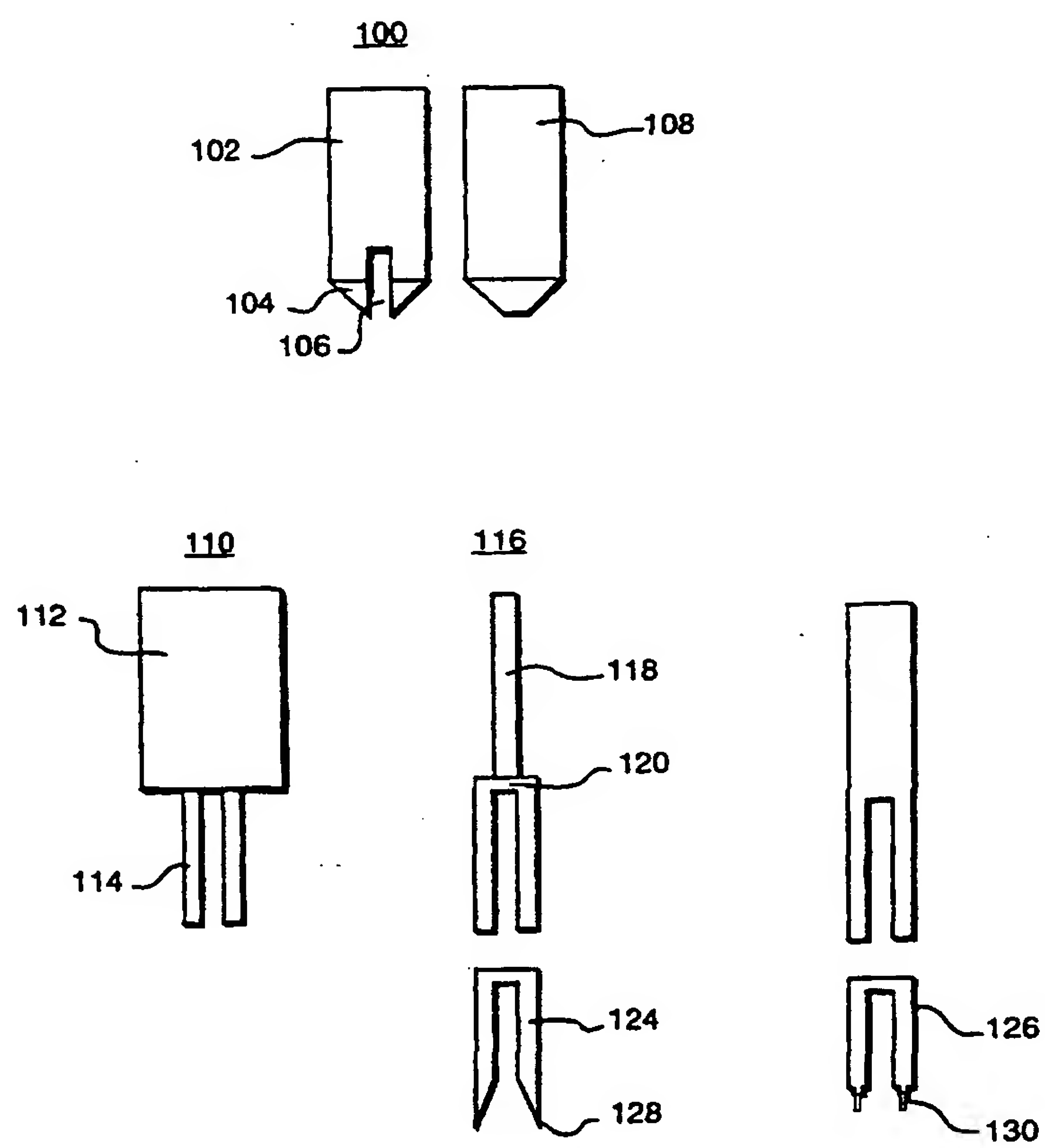
(54) Title: SURFACE TENSION LOADING INERTIAL RELEASE DROPLET DEVICE AND METHOD




**(57) Abstract:** Methods and devices are provided for the delivery of droplets of fluid, small volumes of less than about 500nL, to a receptacle with high precision and low total fluid loss. The methods employ a fork comprising a pair of prongs having inter-prong surface properties and dimensions for loading the small volume, and means for accelerating and decelerating the fork and comprise the steps of: 1) contacting the fork with the liquid, whereby the small volume loads between the prongs by the action of surface tension; 2) moving the fork into liquid transfer relationship to the receptacle; 3) accelerating the fork; and 4) rapidly decelerating the fork at or in proximity to the recipient surface to transfer the droplet.

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# SURFACE TENSION LOADING INERTIAL RELEASE DROPLET DEVICE AND METHOD

## INTRODUCTION

### Field of the Invention

The field of this invention relates to methods and devices for transferring small volumes of fluid with high precision and low fluid loss. The methods and devices of the invention are pertinent to transfer of small fluid volumes for micro-analytical, micro-synthetic and other micro-chemical applications, especially where reagent and/or sample conservation is important as in biochemical and biomedical applications.

### Background

Methods and devices for transferring small volumes of fluid have become more important with the advent of micro-analytical, micro-synthetic and other micro-chemical applications in arts that include the biochemical, biomedical and biotechnological technologies. The phrase "small volumes" is meant to refer to volumes of less than 500 nL, which are termed droplets. The consensus mechanism of existing devices and methods for dropletting is by effecting a positive displacement to either load or unload the material or both.

The size of analyte and other aliquots for micro-chemical manipulations has been rapidly decreasing in recent years as micro-fabrication technologies have improved to permit the intricate fluid transport and handling capabilities required of such devices at ever smaller dimensions and therefore volumes. For example U.S. Patent No. 4,452,899, entitled Method for Metering Biological Fluids, issued June 5, 1984, discloses a method for repeatedly and precisely metering 10 to 300 μL aliquots onto generally planar analysis slides. The lower limit of 10 μL is described in that patent as state of the art, with reference made to an analyzer capable of obtaining a measurable signal with only 10 μL of analyte described in a commonly assigned patent. The operative mechanism for the fluid metering described in U.S. Patent No. 4,453,899 is by the creation of positive pressure to eject and to indirectly create negative pressure for loading by aspiration of the fluid of interest into a disposable tip.

The aliquot volumes for the instant invention are smaller by one to two orders of magnitude. Measuring such volumes sufficiently precisely into a tip by the creation of negative pressure and ejection by positive pressure requires exquisitely precise control of pressure which is economically impractical.

More recently, as disclosed in U.S. Patent No. 5,320,250 issued June 14, 1994, precise control of the momentum of a hammer impacting a diaphragm that deforms has been employed to eject a predetermined amount of a viscous fluid from an adjoining chamber. Fluid being incompressible the pressure at the diaphragm ejects the fluid, which is held in the chamber by its own viscosity. The chamber contains a larger volume than is ejected in fluid delivery, and is refilled from a gas pressurized reservoir after delivery of an aliquot. Thus pressure is the direct mechanism of loading and ejection, and momentum transfer indirectly overcomes viscosity by creating the pressure.

In view of the increasing importance in being able to transfer very small volumes efficiently, accurately and reproducibly, there is substantial interest in developing alternative and improved devices and methodologies to achieve this purpose.

**Relevant Literature**

Literature of interest includes U.S. Patent nos. 4,453,899 and 5,320,250.

## SUMMARY OF THE INVENTION

Methods and devices are provided for the delivery of droplets of fluid, small volumes of less than about 500nL, to a receptacle with high precision and low total fluid loss. The methods employ a fork comprising a pair of prongs having inter-prong surface properties and dimensions for loading the small volume, and means for accelerating and decelerating the fork and comprise the steps of: 1) contacting the fork with the liquid, whereby the small volume loads between the prongs by the action of surface tension; 2) locating the fork and the receptacle in liquid transfer relationship; 3) accelerating the fork; and 4) rapidly decelerating the fork at or in proximity to the recipient surface to transfer the droplet. Various device components are employed with the fork to accelerate and decelerate the fork over a small distance to release the liquid droplet.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates various examples of a fork comprising a pair of prongs;

Figure 2 depicts a liquid transfer fork device with slides with a cylindrical guide; and Fig. 2a is bottom view;

Figure 3 depicts a liquid transfer solenoid activated device;

Figure 4 depicts a liquid transfer pneumatically controlled device;

Figure 5 depicts an alternative embodiment of a liquid transfer pneumatically controlled device; and fig. 5a is a side view of an individual pin; and

Figure 6 depicts a pivot arm carrier and a liquid transfer having an array of liquid transfer forks.

Figure 7 depicts the device incorporating a dampening unit.

## DESCRIPTION OF THE SPECIFIC EMBODIMENTS

In accordance with the subject invention devices and methods are provided for reproducibly and efficiently transferring small droplets of liquid from a source of the liquid, as a substantially integral drop. The volumes of interest are primarily aqueous, although other solvents are also used. The device comprises a liquid transferring unit having two spaced-apart prongs, where the confronting surfaces of the prongs provide for the liquid rising between the two prongs as a result of surface tension, where the volume of liquid taken up or loaded between the two prongs is substantially reproducible. After the device is contacted with the source of liquid, it is removed from the source of liquid and located in relation to the receptacle for the receptacle to receive the liquid. The liquid transfer unit is used in combination with a manipulator, which serves to rapidly accelerate and then decelerate the unit above the receptacle, whereby upon deceleration, the liquid between the two prongs is expelled to be received by the receptacle. The units may be individual units or may be formed as arrays, where each unit may be simultaneously contacted with a source of liquid, removed from the sources of liquid, positioned in relation to a plurality of receptacles and the individual unit or array of units then manipulated to be accelerated and then rapidly decelerated to transfer the individual liquid droplets between the prongs to the receptacles.

The manipulator may be any device, which allows for the rapid acceleration and deceleration of the unit. Accelerations should be at least 0.5 meter/sec, preferably at least 2 meter/sec and will usually not exceed about 10 meters/sec. The acceleration of the drop upon

deceleration will be in the range of at least about 100g, usually at least about 200g and not more than about 2000g, more usually not more than about 1000g. Acceleration may be constant or varied, where the acceleration may increase during the movement of the liquid transfer unit. The considerations about velocity, acceleration and deceleration are that the droplet must be released from the liquid transfer unit and the velocity of the droplet at the time of impact of the droplet on the receptacle surface should not result in extensive spattering or loss of the liquid.

The manipulator may be as simple as a tube in which the unit may be dropped from an appropriate height, where impact of the unit with the floor of the receptacle provides the deceleration, to more sophisticated units, such as solenoids where the unit is magnetic or an electrical coil, pneumatic means, mechanical means, or the like. The particular manner in which the unit is accelerated is not critical to this invention, so long as the acceleration which is achieved is at least the required acceleration for reproducible release of the droplet held in the unit. The deceleration will be designed to fit with the manner of acceleration. Particularly, mechanical impact may be used to provide the rapid deceleration which is required, so that the unit will be stopped above the surface of the receptacle upon which the droplet is deposited. Various designs of flanges, bars, gears, dashpots, snubbers, springs, and the like may be used to stop the movement of the unit after acceleration. The travel distance for the prongs will generally be in the range of 0.5 to 15cm, more usually in the range of 0.75 to 3cm, depending on the force transmitted to the liquid transfer unit, the acceleration achieved by the liquid transfer unit, the rate of deceleration, the nature of the liquid to be transferred, the force required to expel the liquid held between the prongs, the nature of the container, etc. The closest distance the prongs will come to the receptacle, normally the floor of the receptacle or the top of the meniscus of any liquid in the receptacle, rather than the lip of the receptacle, will be in the range of about 0.02 to 3cm more usually in the range of about 0.1 to 1cm, except when the liquid transfer unit impacts the floor, where the distance is zero. The container receiving the droplet may be dry or have a liquid cushion, depending on the purpose for which the liquid transfer is being made.

The manipulator will have a small footprint when used with a single liquid transfer unit and will usually be smaller than the area covered where an array of liquid dispensing units are employed, although the footprint size will be of less significance in this case. For example, the area occupied by the manipulator may only be about 1 by 1cm to about 10 by 15cm (about 1 to 150cm2), when used with a microtiter 96 well plate and would have to be smaller if used with a

384 well plate. As the space permitted for the manipulator becomes increasingly smaller, an array of liquid dispensing units will be employed using one or a few manipulators to expel the droplets.

The units may be relatively small, in view of the small volumes, which are involved. The volume to be transferred will generally be less than about 500nl, generally in the range of about 10 to 250nl, more usually in the range of about 10 to 150nl. While the subject devices find particular application with very small volumes, the subject devices may also be used for applications with larger volumes, up to about 5μl, particularly where the liquids do not pipette accurately with air-displacement pipetters. This can include "stringy" fluids, such as capillary electrophoresis separation media or protein solutions. The length of the prongs will generally be in the range of about 0.25 to 10mm, more usually in the range of about 0.5 to 5mm, where at least about 25%, more usually at least about 50%, of the volume enclosed by the prongs will be occupied by the liquid. The spacing between the prongs will generally be in the range of about 5μ to 500μ, more usually in the range of about 10μ to 300μ, where the spacing may be constant or tapered, so that the opening will be larger than the spacing above the opening. If tapered, the taper may be only a portion of the way to the top of the prongs or may extend the entire length of the prongs. Where only a portion, it will usually not extend beyond 50% of the length of the prongs. The internal surface of the prongs may be any regular surface, normally smooth, and may be flat, concave, angular, e.g. having two sides defining an acute angle, etc. If one considered the circumference by filling in the spacing between the prongs, the O.D. of such cylinder would generally be in the range of about 0.1 to 1.5mm, more usually 0.25 to 1mm. The prongs may be longer than required for the volume to be transferred. In this case, a cap or barrier may be placed between the prongs to limit the height to which the liquid droplet will rise. The volume enclosed by the prongs may be adjustable, so that an individual pin may be adjusted to provide different volume capacities. The barrier may physically limit the volume the liquid can wick into or may be a surface treatment (e.g. hydrophobic for aqueous liquids) preventing the fluid from wicking above a desired point between the prongs. The changes in volume capacity may be as a result of moving a cap at different distances from the opening at the ends of the prongs, having a threaded rod which extends into the space between the prongs, which may be varied as to the distance from the end of the threaded rod to the end of the prongs, providing for an expansion screw, so as to change the taper between the prongs, or any other mobile element

which can change the distance between the opening at the bottom of the prongs and the height to which fluid can rise.

The prongs may be portions of a cylinder, where the inner surface is round, hemispherical where the inner surface is flat, or other configuration. The outer surface may be round, flat, or other configuration, where the outer surface should minimize the amount of the liquid which clings to the outer surface. The inner surface of the prongs will be selected to provide the appropriate surface tension for the liquid to rise to the proper level between the prongs. Since, for the most part, the solutions of interest will be aqueous, the inner surface will by hydrophilic. A wide variety of hydrophilic compositions may be used structurally or as hydrophilic coatings, such as silicates, e.g. quartz, glass, bioglas, etc., polymers, such as poly(vinyl alcohol/acetate), poly(acrylic acid/ester), poly(hydroxyethyl acrylate or methacrylate), etc. or a hydrophobic material may be coated, covalently or non-covalently with a hydrophilic material, e.g. proteins, saccharides, amino- or hydroxy-substituted dendrimers, polyoxyalkylenes, etc. For hydrophobic materials, metals, ceramics and hydrophobic polymers may be employed, such as polystyrene, polyimides, polyacrylates, polyaldehydes, polyolefins, e.g. polyethylene and polypropylene, and the like. Alternatively, the hydrophilic materials may be coated with hydrophobic coatings, such as waxes, oils, hydrophobic polymers, and the like.

The prongs will be attached to a support to hold the prongs in confronting position. The support will be designed in accordance with the nature of the manipulator. The support may be a cylinder, rod, having a circular, rectangular or other cross-section, two rods held together by bridges, serpentine, spiral-shaped, etc. The support is characterized by holding the prongs in apposing position and mating with the manipulator to provide the desired kinematics. Motivation for the acceleration may be gravity, pneumatic means, electrical means, mechanical means, or combinations thereof. Thus one may use pressure-activated pistons, springs, solenoids, motors and gears, and the like.

In the simplest exemplification of the subject invention, and primarily for illustration of its effectiveness, the method and device employ a fork comprising a handle and a slotted pin that slides inside a sleeve. This exemplification may be modified by employing an arm with a fork harness that positions the fork in the XY plane while permitting it to move upwards in the Z direction so that when the arm causes the fork to contact the target or recipient surface, the fork

can bounce off the surface before being lifted away by the arm as it is raised back up after the contacting.

The fork may have a slot that is small enough in the Z or upwards dimension so that the top of the slot prevents more than a specific amount of fluid from entering the inter-prong space. Alternatively the amount of fluid loaded may be dictated by the equilibrium of the surface tension, external pressure, and column weight. An analogy may be drawn to a simple capillary tube. There will be a column height for a given fluid and inside surface that depends upon the surface tension.

In a capillary tube, the column hydrostatic pressure, is given by the equation

$$\mathbf{p} = \rho^*\mathbf{g}_e{}^*\mathbf{h},$$

where $\rho$ is the density of the liquid $g_e$ is acceleration due to gravity, and **h** is the column height. The column hydrostatic pressure must equal the pressure difference between a point just under the meniscus and the external pressure resulting from the surface tension, given by:

$$\Delta\mathbf{p} = 2^*\gamma/\mathbf{r}$$

where **r** is the radius of the tube and $\gamma$ is the surface tension.
The column height equals the difference between pressure, thus:

$$\mathbf{h} = 2^*\gamma/\rho^*\mathbf{g}_e{}^*\mathbf{r}.$$

Thus, for a capillary tube having radius r, assuming a hemispherical surface, the column height, given by equating the column pressure and surface tension pressure difference is inversely proportional to the separation between the capillary walls. Atkins, Physical Chemistry, 6th Ed., 1998, W.H. Freeman & Co., New York.

For two separate parallel prongs, the meniscus surface is closer to hemicylindrical, but a similar relationship may be shown:

$$\rho^*\mathbf{g}_e{}^*\mathbf{h}^*\mathbf{D}^*\mathbf{W} = 2^*\mathbf{W}^*\gamma,$$

where **D** is the separation between the prongs and **W** is the depth of the slot formed by the prongs, thus $\rho^*\mathbf{g}_e{}^*\mathbf{h}^*\mathbf{D}^*\mathbf{W}$ represents the column weight and $2^*\mathbf{W}^*\gamma$ represents the upward pull of surface tension assuming a zero contact angle. Thus for the parallel prongs:

$$\mathbf{h} = 2^*\gamma/\rho^*\mathbf{g}_e{}^*\mathbf{D}.$$

Substituting **D = 2r** yields:

$$\mathbf{h} = \gamma/\rho^*\mathbf{g}_e{}^*\mathbf{r},$$

thus confirming that the parallel prongs will support a smaller column height than a closed tubular capillary having the same separation between the walls, and that the height is inversely proportional to the inter-prong distance.

If the slot is capped at a height less than the column height, as in the exemplified embodiment, the cap determines the amount loaded. Technically, once the meniscus disappears surface tension does not exist, and the force and corresponding energy required to dislodge the droplet is against surface adhesion. Atkins, Physical Chemistry, 6th Ed., 1998, W.H. Freeman & Co., New York. For the purposes of the instant invention, surface tension contemplates surface adhesion in addition to classical surface tension. If the slot is not capped at a height less than the column height, choosing or setting the volume to be transferred may be effected by setting the inter-prong distance. As different solutions will have different values of $\gamma$, different volumes will be measured by the same inter-prong distance and geometry. But for fluids having values of $\gamma$ that differ only slightly, such as most aqueous solutions, any difference will be insignificant. From the preceding the additional conclusion that the capped column is more difficult to dislodge than the uncapped column because there is more adhesive surface to fluid volume, and thus a greater adhesive force to weight ratio in the capped as opposed to uncapped transfer element. Experiments have shown that sufficient deceleration can easily be generated to successfully dislodge substantially all fluid from such a capped column.

In addition to allowing setting the volume loaded by setting the inter-prong distance, for the uncapped column, the preceding theoretical analysis permits another conclusion of practical import by permitting reduction of the difficulty of dislodging the droplet by gradually increasing the inter-prong distance in the direction of the tip of the transfer element. For example tapering of the prong so that the inter-prong distance increases towards the tip reduces the ratio of upwards surface tension force to weight, facilitating transfer of the droplet with less acceleration at impact.

Generally, other means than gravity are employed for the kinematic acceleration of the fork. In one such embodiment the method and device employ a piston or plunger assembly comprising a magnet or solenoid attachment connected to the prongs that is accelerated by the action of the solenoid magnetic field on handle of the fork. The solenoid acts on the handle of the fork by the action of the solenoid magnetic field on a magnet or by the action of the solenoid on an electric coil (voice coil) or other electromagnet. A stop is provided to terminate the

movement of the fork with the prongs at a position close to the targeted surface to allow the release of the droplet without the prongs touching the target surface. Another variation of the preferred embodiment substitutes a pressure apparatus in the piston assembly to impel the fork towards the stop. A spring may be used as the actuating mechanism. The spring could be mechanically compressed and captured, then released to accelerate the pins. Alternatively, the deceleration may be effected without any impact or collision between two solid objects, as when the fork is both accelerated and decelerated by magnetic or pneumatic means. A multiplicity of the elements described in the preceding embodiments may be employed to transfer droplets of the same or different liquids simultaneously.

The device is designed to have solely vertical motion and minimize lateral motion. Various ways may be used to require that the fork unit be constrained from moving in the horizontal direction, which could result in vibration and shaking. Therefore, the prong holding unit will generally be housed in a close fitting cylinder, where lateral movement is prevented. By providing for close fitting tolerances and lubricated surfaces, or reducing the area of contact between the prong holding unit and the housing, one can provide for substantially unimpeded vertical movement, while still orienting the prong holding unit in a confined trajectory. The surface area contact may be reduced by using rods with fins, projections, circumferential guides, etc.

When transferring the droplets, the liquid may include a standard to indicate the volume that was actually transferred. Thus, a fluorescent or light-absorbing dye may be used, where the total volume will be subject to detection or a known portion of the total volume subject to detection. In this way, where a plurality of droplets are being transferred, particularly where results are being compared, one can use the relative volumes to normalize the final results.

To minimize the probability that extraneous, uncontrolled fluid dropets adhere to the outside surface of the pin after withdrawal from the source liquid, it may be desirable to only immerse the pin to not more than about 50% of the fork length below the meniscus of the source fluid. This is best accomplished by implementing a liquid-level sensing system, which senses the actual liquid meniscus and immerses the fork a predetermined distance below the meniscus. As source liquid levels in adjacent reservoirs on a 96 or 384 well plate can vary substantially, it may be desirable to individually control each pin. It will be appreciated that established techniques, such as sensing the change in capacitance of a probe as it is immersed into a liquid,

can be applied to sense the liquid meniscus location. Other means of sensing the fluid level include ultrasonic measurement, optical means, fiber-optic means and measuring the physical damping of a vibrating probe as it is immersed into aliquid. It will also be appreciated that combining, for each pin, such form of level sensing, electrically controllable actuation, e.g. pneumatics or solenoids, and a controlling computer allows a very sophisticated liquid level sensing system to be implemented providing individual and independent liquid level sensing for each pin actuator in an array of pins.

For further understanding of the invention, the figures will now be considered. In Fig. 1, various configurations of fork devices are depicted. A device 100 has a cylindrical body 102 and tapers at one end 104. The tapered end 104 has slot 106 extending across the tapered end 104, where the slot 106 has flat apposing walls. The view of the rod 100 at turned 180° 108 shows a solid tapered end. Various other forms of the device fork may be employed in relation to the manipulator, which is used. Device 110 has body 112 and spaced apart prongs 114, which are within a smaller circumference than the body 112. By way of contrast, device 116 has body 118, platform 120 and prongs 122, where the prongs 122 are outside the circumference of the body 118. Prongs 124 and 126 have tapered ends 128 and 130, respectively, where the taper of the ends 128 is limited to the inside of the prongs 124, so that the outer circumference is the same the entire length of the prongs, while prongs 126 are tapered on the inside and outside, so as to form a cone. Platform 120 may be slanted to facilitate rinsing of the device during subsequent cleaning.

In Fig. 2, a liquid transfer fork device 130 has cylindrical body 132 and guiding spacers 134. The guiding spacers can be used in a variety of ways. The guiding spacers 134 may provide for frictionless travel in a cylindrical or rectangular or other regular shaped housing, may be magnetic, where cylindrical housing 136 is encompassed by a solenoid, or may support a compressed spring, so that upon release of the guiding spacers 134, the fork device 130 is driven downwardly through the cylinder 136 to be stopped by stops 138. The fork device is tapered at one end 140, into which is cut a slot 142. The slot 142 is shown filled with liquid 144, when mounted in housing 136. In operation, the device 130 in housing 136 is placed in its lowered position against stops 138 and contacted with the liquid to be transferred. Liquid 144 is taken up into slot 142 and the housing 136 with the device 130 moved to be positioned over receptacle 146. The device 130 is then rapidly propelled toward receptacle 146, so that when it hits the

stops 138, it is traveling at a velocity of about 500 to 2000 meters/sec. The substantially instantaneous deceleration results in the liquid 144 being propelled toward receptacle 146 and being deposited on the surface of receptacle 146 without significant splashing. In Fig. 2a the cylindrical body 132 has slot 142 extending the diameter of the cylindrical body 132.

In Fig. 3 a device employing a solenoid and spring for acceleration and deceleration is depicted. The device 300 has the fork unit 302 and the solenoid housing unit 304. The fork unit 302 has magnetic cylindrical body or barrel 306, cover 308 and prongs 310, in which liquid 312 is penned in. The barrel 306 is at a raised position due to cover 308 resting on spring 314, which is in the resting or expanded state. The housing 304 has a solenoid coil 316 surrounding cylinder 318, which surrounds the barrel 306. The barrel and the internal surface of the cylinder 318 may be coated with a low friction material, such as a polyfluorinated polymer, to allow for rapid movement of the fork device unit through the cylinder of the housing. Alternatively, various lubricants may be used to reduce the friction during the descent of the barrel 306. In operation, the device 300 is moved to be in contact with a source of liquid, whereby the liquid moves between the two prongs, based on the surface tension between the liquid 312 and the internal surface of the prongs 310. As shown, the liquid 312 has moved to the top of the slot between the prongs 310. The device 300 is then moved to be in juxtaposition to the receptacle 320. The solenoid 318 is then activated accelerating the barrel 302 downwardly, where spring 314 is compressed by cover 308. The barrel 302 is stopped by platform 322 with the force being cushioned by spring 314. The liquid 312 is expelled from between the prongs 310 to form droplet 324 on receptacle 320. The solenoid 318 is then deactivated, whereby the spring 314 expands to restore the barrel 302 to the raised position in readiness for the next transfer. Where different liquids are transferred, the prongs would be washed both internally and externally by any convenient means, such as immersion in an appropriate solvent or solution, particularly one which is volatile, so as to dry rapidly and leave no residue. Alternatively, the prongs may be sprayed with a cleaning solution, sonically cleaned in a cleaning medium, or may be cleaned by other conventional cleaning methods.

In Fig. 4, a number of different pneumatic approaches are illustrated. The systems may rely solely on pressure to move the fork unit or may combine pneumatic means with mechanical means. In device 400, a cylindrical housing 402 has a ceiling 404 and a floor 406. Mounted in the cylindrical housing 402 is liquid transfer unit 408. The liquid transfer unit 408 has a piston

410 to which is attached prongs 412. The bottom 414 of the rod 410 engages spiral spring 416. Extending into and through ceiling 404 is a gas supply pipe 418, which supplies gas to the region 420 above the piston 410. The volume of the space above the piston 410 should be minimal, so that the gas introduced into the region 420 can provide for maximum acceleration of the piston 410 over as short a time as possible. Pressures to be applied will generally be in the range of about 5 to 50psi. The acceleration should provide a velocity at deceleration of at least about 0.5m/sec.

Upon introducing gas at high pressure, the region 420 expands, driving piston 410 downwardly at a high velocity compressing spring 416 and extending the prongs 412 out of the housing 402 toward a receptacle, not shown. As the momentum created by the gas pressure is overcome by the opposing force of the compressed spring 416, the piston will rapidly decelerate and liquid retained between the prongs 412 will be rapidly expelled. The pressure in region 420 may then be released through gas supply pipe 418 and the compressed spring 416 expanded to restore the fork device unit to a position for loading with liquid and repeating the process.

While a spiral spring has been exemplified, other resilient or compressible devices may be used to decelerate the liquid delivery unit and restore it to a liquid receiving position. These include elastomeric pads, leaf springs, compression springs, dashpots, snubbers, shock absorbers, etc. While only one gas supply conduit has been depicted, one could have two conduits, one for supply and one for release of the pressurized gas. By appropriate valving, gas could be introduced into the region above the piston and upon release of the liquid, the pressure in the region would be dissipated through the other conduit. Rather than using a spring to restore the delivery unit to a liquid receiving position, vacuum can be applied to the enclosed chamber above the piston.

As depicted in Figure 7, the mechanical stop can be configured as a dampening unit for directing impact shock away from the instrument. Similar to the other disclosed embodiments, the connecting rod 712 is attached to a pneumatic piston assembly 706 and the sample dispensing pins 728, the assembly 706 and dispensing pins 728 being at opposite ends of the connecting rod. The stationary portion of the assembly 706 is usually attached directly to the instrument frame although variations of this configuration are possible. The pneumatic piston assembly usually will incorporate o-rings, piston rings, or other known devices for maintaining a pressure resistant seal around the piston 708 and the connecting rod 712. Oil or Teflon can also

be used in the assembly to minimize the detrimental effects of friction. A head plate 714 is fixed to the connecting rod 712 between the pneumatic assembly 706 and the pins 728. Between the impact plate 714 and the pins 728, the connecting rod freely passes through a dampening mass 718 whereby the connecting rod is free to move as directed by the pneumatic assembly 706. Preferably, the mass 718 comprises a guide for the rod 712 so as to restrict unwanted lateral movement. The dampening mass 718 is mounted on dashpots or snubber assemblies 720 which are in turn connected to the instrument frame 726. Various known configurations are available for the dashpot or snubber assemblies including the depicted combinations of shock absorbers 722 and springs 724 whereby upon actuation, the springs operate to return the device to its static position. The shock absorbers or dashpots basically comprise a pneumatic or hydraulic piston unit configured to absorb and dissipate the kinetic energy through mechanical means such as pressure, friction, and heat. These absorbers or dashpots are commercially available and known in the art, for instance Shock Absorber Part No. OEM-1.0-(B) manufactured by Enidine Inc. Resilient pads 716 are also provided at the interface between the impact plate 714 and the dampening mass 718. Electromagnet 700 acts upon the cover plate 702 attached to the piston assembly 706 to draw back the piston 708 creating both a pressurized portion 704 and vacuum portion 710 in the cylinder, thereby creating a pneumatic spring. When electromagnet 700 is deactivated, piston 708 is accelerated, driving the impact plate 714 downward wherein it strikes the dampening mass 718 and effectuates an instantaneous deceleration of the sample dispensing pins 728. To ensure that the sample pins 728 are subjected to the necessary deceleration required for droplet ejection into receptacle 730, the moving mass 718 has an effective mass that is generally in the range of 1 to 50 times the mass of the impact plate 714, preferably 5 to 20 times the mass of the impact plate, more preferably 10 times the mass of the impact plate. In combination with the snubber assemblies 720, the pads 716 also help to control and alleviate the forces from the impact plate striking the moving mass. Through this cascade of energy transfer, the generated shock is effectively dampened.

Rather than use mechanical means for deceleration, one could use pneumatic means for both acceleration and deceleration. In Fig, 4b, the liquid dispensing device 450, has the liquid dispensing unit 452 comprising a piston 454 narrowing to a neck 456 from which a pair of prongs 458 extend. The liquid dispensing unit 452 is housed in a cylindrical housing having ceiling 462 and floor 464. A pair of gas conduits 466 and 468 have three-way valves 470 and

472, respectively. Similar to the previous device, the conduits 466 and 468 serve to provide a rapid influx of air into the region 474 above the piston 454. Extending through the floor 464 are two conduits, 476 and 478, fitted with three-way valves 480 and 482, respectively. The valves will usually be electrically controlled, so as to allow for rapid opening and closing of the valves during the liquid dispensing operation. In performing the liquid dispensing, the device 450 would be placed with the prongs 458 in contact with a liquid source, whereby the liquid would rise and be loaded between the prongs 458 in an amount determined by the density and viscosity of the liquid and the surface tension between the liquid and the walls of the prongs 458. The liquid filled device 450 would be moved or the source of the liquid replaced with the receiving receptacle, so that the now liquid-filled prongs would be over the receptacle loaded for liquid transfer. The conduits 466 and 468 would be connected to a highly pressurized plenum. Conduits 476 and 478 are also connected to a plenum, which is at atmospheric pressure. Upon the device being in appropriate position in relation to the receptacle, valves 470, 472, 480 and 482 would be simultaneously opened to the plenums. The pressurized gas from the pressurized-gas plenum would be released into region 474 driving the piston 454 downwardly at high velocity, whereby the neck 456 would form a gas seal between the neck 456 and the floor 464. The gas in the region 484 would be driven into the conduits 476 and 478 and then into the atmospheric pressure plenum, not shown, where the pressure would become somewhat elevated. The air captured in the region 484 would serve to cushion the piston 454 against impact against the floor 464. By appropriate choice of volumes and pressures, having a piston with a smaller diameter at the top, where the chamber 474 is reduced in size to accommodate the rod, and the gas capacity of the conduits, the desired velocity of the fluid dispensing unit may be achieved for efficient fluid transfer.

As depicted in Fig. 5, a pneumatic device may be used in an alternative way. The device has a liquid dispensing unit 502 comprising a piston 504, a connecting rod 506 to which is bound liquid dispensing pin plate 508. Plate 508 can preferentially be manufactured from a light, stiff material, such as a graphite-epoxy composite to minimize the impact load of the plate on the supporting structure during deceleration. Coupled to plate 508 are liquid dispensing pins 510. The plate 508 may have any pattern of liquid dispensing pins to integrate with the receptacle. Where the receptacle is a microtiter 96 well plate, the pins may be oriented in relation to the wells of the plate in an 8 x 12 arrangement. Where the receptacle has a different pattern, the

layout of the plate 508 may be similarly organized. It will be appreciated that the pins must be substantially parallel to the direction of motion, to eliminate lateral whipping during deceleration. In certain configurations, it may be desirable to permit pivoting of the individual pins in an elastomeric (damped) support to permit the pins to self-align to a perfectly vertical position during deceleration. In such an embodiment, a two-stage deceleration is desirable – a first, moderate deceleration aligns the pins parallel to the direction of motion; and a second, severe deceleration dislodges the liquid droplets.

As depicted in Fig. 5a, the dispensing pins 510 have a threaded head 512 connected to prongs 514 with orifice 516 surrounded by tapered end 518. A liquid cap or stop 519 controls the amount of liquid which is drawn up between the prongs 514. The threaded pins 510 screw into mating threaded female members 520. While a threaded attachment has been illustrated, any means for firmly fastening the pins to the plate may be employed, such as spring fasteners, locking fasteners, such as rods with projections, where the projection may be turned into a receiving channel, etc. The particular choice of attaching the pins to the plate will be primarily a matter of economics and convenience.

By having exchangeable pins, many advantages ensue, in that the amount of liquid to be transferred can be changed at individual sites on the plate or for the entire plate. Broken or damaged pins may be readily replaced at a particular site. The pins may be individually removed to be thoroughly cleaned, sterilized, or treated in a particular manner for a particular application.

The piston has circular grooves 522 holding o-rings 524, to provide a leak-proof seal, where the o-rings are lubricated or are of a low-friction material, e.g. Teflon®, to allow for ease of movement in the housing 526. It will be appreciated that a pneumatic system without o-ring seals can also be implemented. Such a system would rely on carefully engineered clearances between the pistons and their cylinders to create an almost frictionless air-bearing between the piston and the cylinder, allowing substantially lower actuation pressures. The housing 526 has conduit 528, which is connected through three-way valve 530 to conduit 532 and vent 534. Conduit 532 is connected to high-pressure pump 536. In addition, housing 526 has stops 538, which support resilient pads 540. Plate 508 is held in place by electromagnets 542. Plate 508 may be fabricated of a ferromagnetic material or have ferromagnetic pads 544, which are held by the electromagnets 542. In operation, the liquid dispensing pins 510 will be placed in contact with a source of liquid for one or more of the pins. After loading the pins, the device 500 or

receiving receptacle may be moved into position for transfer of liquid to the receptacle. Then, the three-way valve 530 is turned to connect conduit 532 with conduit 528. The pump 536 is turned on and the region above the piston 546 is pressurized to the desired pressure, while the electromagnets 542 hold the liquid dispensing unit 502 in place. When the desired pressure has been achieved, the electromagnets 542 are deactivated releasing the plate 508. The gas pressure in the region 546 above the piston drives the liquid dispensing unit 502 downwardly at a high velocity until the piston engages the resilient pads 540 and is stopped with expression of the liquid in the pins 510 onto the receiving receptacle. The three-way valve 530 is then turned to connect 528 with vent 534 to release the pressurized gas in region 546 and the magnets activated to retrieve plate 508, restoring device 500 to a state of readiness to repeat the process.

The liquid dispensing device may be mounted on a moveable and pivotable arm to allow the liquid dispensing device to move in the Z-direction, as well as in the X-Y direction. In Fig. 6 is a diagrammatic depiction of a device 600. The device has a liquid dispensing apparatus 602 and a pivoting carrier 604 unit. The carrier 604 has a base 606, a motorized bearing 608 and a stand post 610. The post 610 is journaled in bearing 608, so as to be able to be turned by bearing 608 about the axis of the post 608. Mounted on post 608 is control swivel bearing 612. Horizontal arm 614 extends between control swivel bearing 612 and swivel bearing 616. Swivel bearing 616 provides that the liquid dispensing apparatus remains orthogonal to the receptacle 618, so that liquid is dispensed perpendicularly to the plane of the receptacle, where the receptacle will normally be oriented horizontally. The liquid dispensing apparatus comprises a housing 620 to which the swivel bearing 616 is attached, a fork liquid dispenser comprising a piston 622, a connecting rod 624, a pin holding plate 626 and liquid dispensing pins 628. In addition, a control box provides for control of the movement of the piston 622 by any convenient means, particularly those which have been described previously. By use of the carrier 604, the liquid dispensing apparatus may be moved in juxtaposition to a source of liquid, e.g. samples. When over the source, the liquid dispensing apparatus may be lowered to be dipped into the reservoirs holding the liquids and the liquids allowed to rise into the liquid dispensing pins. The liquid dispensing apparatus may then be raised and moved to a receiving receptacle or the liquid source replaced with the receiving receptacle. The liquid dispensing apparatus may then be

positioned at the appropriate height and the piston accelerated and the decelerated to release the liquid in the pins.

The subject methods and devices find use in transferring small volumes to microtiter plates for diagnostics, cell biology, DNA analysis, including sequencing, detection of mutations and single nucleotide polymorphisms, and the like. In combination with miniaturized devices, such as microfluidic devices, combinatorial chemistry, etc., the subject devices permit small volumes to be manipulated and transferred from one site to the next.

The following examples are offered by way of illustration and not by way of limitation.

## EXPERIMENTAL

### Example 1

A circularly cylindrical slot pin, diameter 0.47 mm, slot width of 0.152mm, and a slot depth of 1.436mm, made of stainless steel, having a nominal calculated slot volume of 100 nL was mounted on the plunger of a solenoid STA195203-13, nominal 24V. The plunger had a flat bottom surface and was stopped by a flat seat. The voltage used was 80V. The distance traveled by the tip of the pin was about 10mm and the distance from the floor of the receptacle at the moment of release was about 1mm. The speed of the tip was about 3m/sec. A 96-well microtiter well plate was used as the receptacle, with the liquid dispensed into the second and third rows. A100μM fluorescein solution was prepared with HEPES buffer as the dispensed liquid. A fluorescence-volume graph was prepared to determine the volume from the observed fluorescence. RFU means relative fluorescence units, which is an arbitrary scale. The volumes transferred are set forth in the following table and indicate efficient reproducible transfer of the nominal volume. The mean result was 83.231, standard deviation was 6.573 and the CV% was 7.9

| Wells | RFU | Result |
|---|---|---|
| A2 | 10.143 | 80.280 |
| A3 | 11.378 | 90.061 |
| B2 | 11.232 | 88.904 |
| B3 | 9.493 | 75.128 |
| C2 | 10.743 | 85.033 |
| C3 | 10.778 | 85.309 |
| D2 | 10.944 | 86.623 |
| D3 | 10.526 | 83.314 |
| E2 | 10.123 | 80.118 |
| E3 | 9.630 | 76.217 |
| F2 | 10.991 | 86.994 |

| | | |
|---|---|---|
| F3 | 9.370 | 74.157 |
| G2 | 10.242 | 81.065 |
| G3 | 10.062 | 79.640 |
| H2 | 12.674 | 100.326 |
| H3 | 10.103 | 79.961 |

All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporate by reference.

The invention now having been fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method for transferring small volumes employing a device comprising two confronting prongs defining a volume between the prongs sufficient for the volume to be transferred and inner surfaces providing surface tension to raise the volume to be transferred between said prongs, said method comprising:

contacting said prongs of said device with a liquid and loading said liquid into said prongs;

locating said device in juxtaposition to a recipient receptacle; and

accelerating and decelerating said device to expel said liquid from between said prongs and into contact with said recipient receptacle.

2. A method according to Claim 1, wherein said liquid is hydrophilic and said surfaces are hydrophilic.

3. A method according to Claim 1, wherein said acceleration and deceleration employ mechanical means.

4. A method according to Claim 3, wherein said mechanical means comprises a spring.

5. A method according to Claim 1, wherein said acceleration and deceleration employ electrical means.

6. A method according to Claim 5, wherein said electrical means employs an electromagnet external to said device and said device comprises a magnet or electromagnet.

7. A method according to Claim 1, wherein said deceleration results from impact of said device with a stop.

8. A method according to Claim 7, wherein the shock from said impact is dampened.

9. A device for transferring a liquid volume of less than about 500nl, said device comprising:

a liquid transfer unit comprising:

two confronting prongs having inner surfaces to provide surface tension and a volume between the prongs sufficient to transfer a predetermined volume of less than about 500 nl;

a holder for holding said confronting prongs in fixed position;

a kinematic device comprising:

a housing for receiving said liquid transfer unit;

means for accelerating said liquid transfer unit in said housing without significant lateral movement; and

means for rapidly decelerating said liquid transfer unit.

10. A device according to Claim 9, wherein said holder is a rod to which said prongs are attached at one end of said rod.

11. A device according to Claim 9, wherein said prongs are tapered.

12. A device according to Claim 9, wherein said means for accelerating comprises a solenoid external to said liquid transfer unit and said liquid transfer unit comprises a magnetic holder.

13. A device according to Claim 9, wherein said means for decelerating comprises a dampening unit.

14. A device for transferring a liquid volume of less than about 500nl, said device comprising:

a liquid transfer unit comprising:

two confronting prongs having internal hydrophilic surfaces providing surface tension with a polar medium, wherein the volume between said prongs and said surface tension with said polar medium will result in a volume of less than about 500nl being loaded between the prongs;

a holder for said prongs comprising a bar to which said prongs are attached at one end, said prongs being within the circumference of said holder;

a manipulator device for accelerating and rapidly decelerating said liquid transfer unit comprising:

a housing into which said liquid transfer unit fits;

accelerating means for rapidly accelerating said liquid transfer over a short distance; and

decelerating means for rapidly decelerating said liquid transfer unit for discharging liquid between said prongs held by said surface tension.

15. A method for transferring small volumes not exceeding about 5µl of liquids difficult to dispense with an air-displacement pipette employing a device comprising two confronting prongs defining a volume between the prongs sufficient for the volume to be transferred and inner surfaces providing surface tension to raise the volume to be transferred between said prongs, said method comprising:

contacting said prongs of said device with a liquid and loading said liquid into said prongs;

locating said device in juxtaposition to a recipient receptacle; and

accelerating and decelerating said device to expel said liquid from between said prongs and into contact with said recipient receptacle.

16. A device for transferring liquid volumes of less than 500 nl, said device comprising:

a connecting rod having first and second ends; a piston assembly for driving said rod attached to said first end; a liquid dispensing array attached to said second end; an impact plate fixed to said rod between said piston assembly and said array; a mechanical stop aligned for impact with said impact plate in a configuration for decelerating said array wherein droplets of liquid are dispensed from said array.

17. A device according to Claim 16 wherein said mechanical stop comprises a dampening mass mounted on one or more dashpot assemblies.

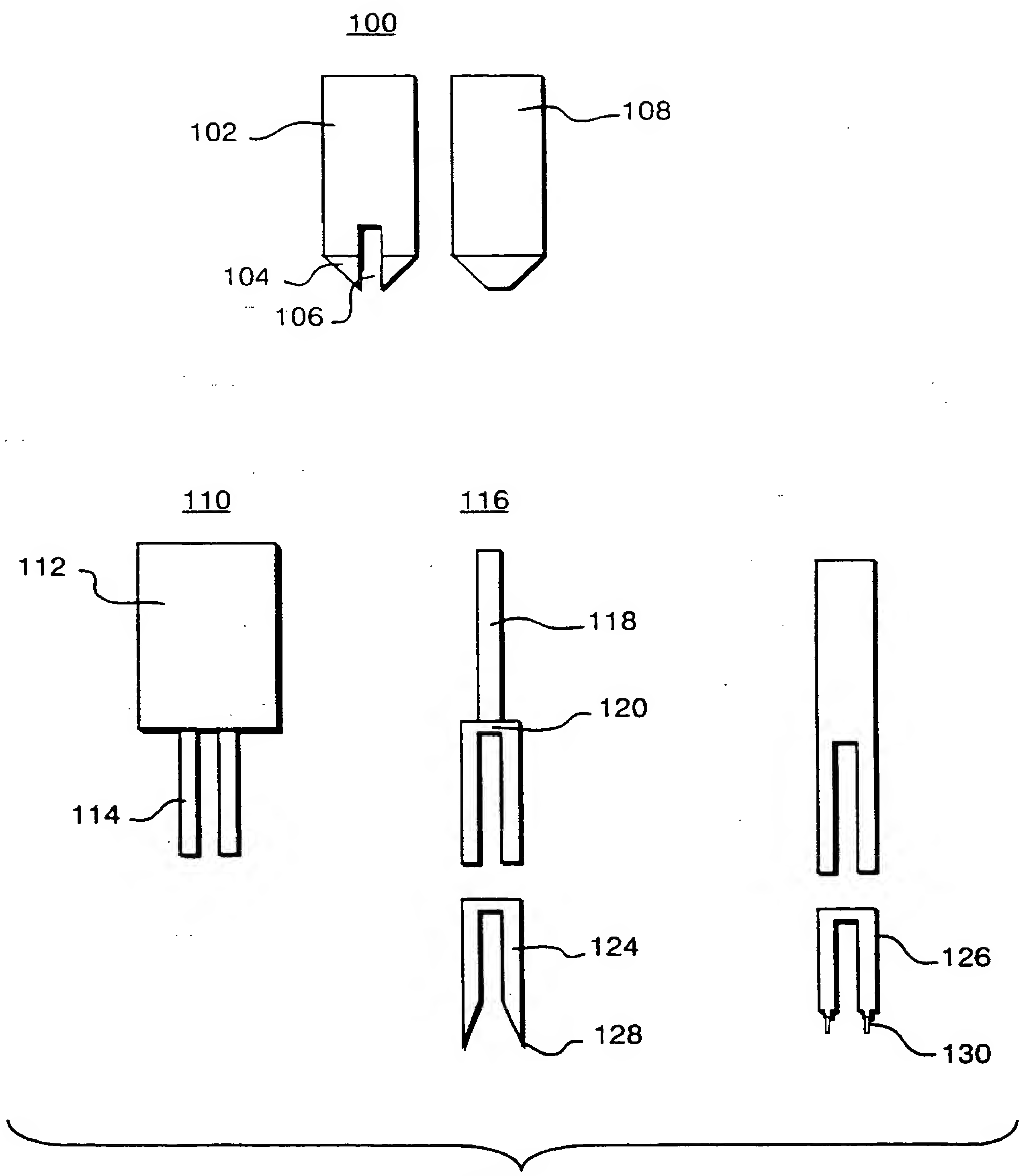


FIG. 1

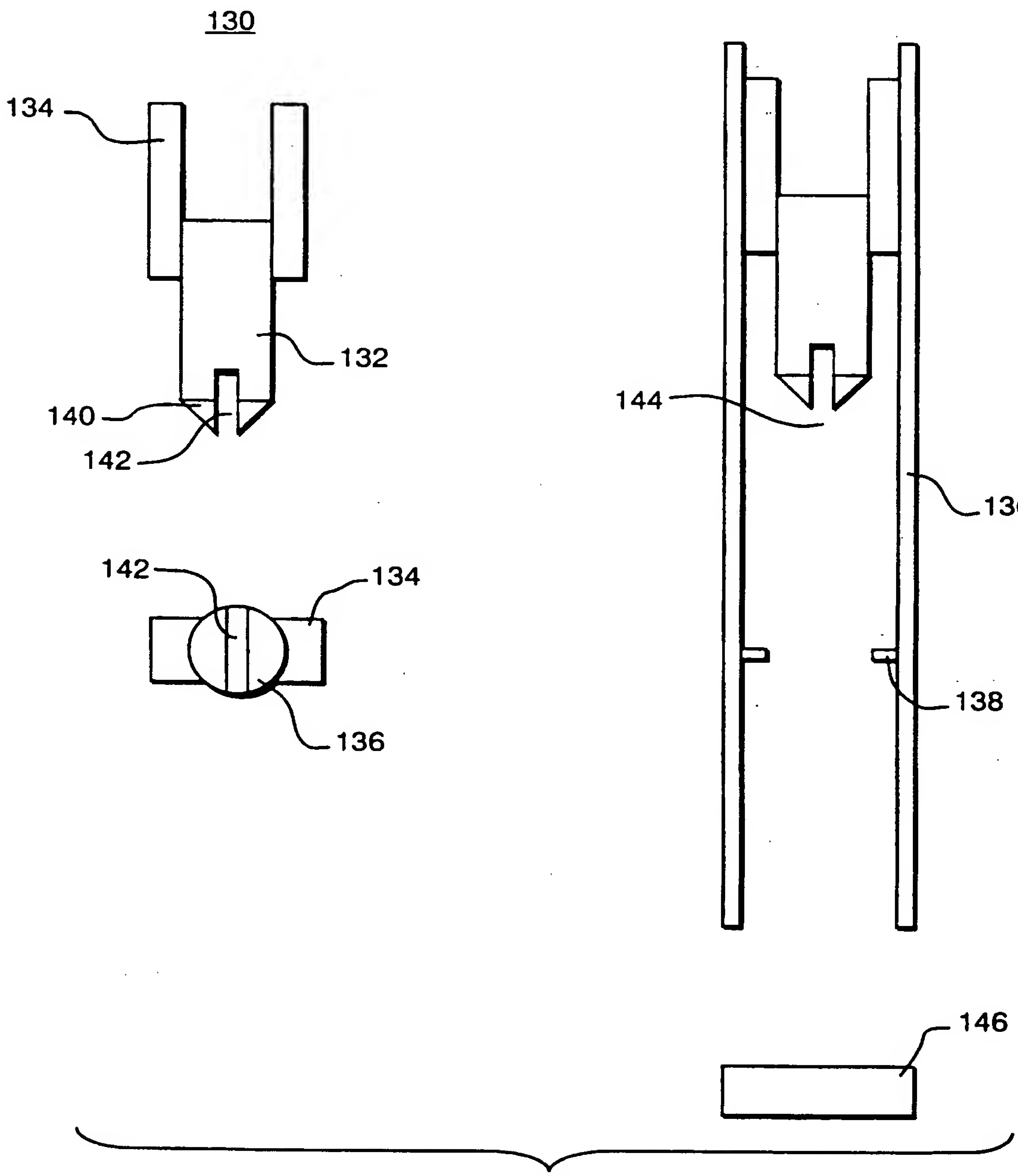


FIG. 2

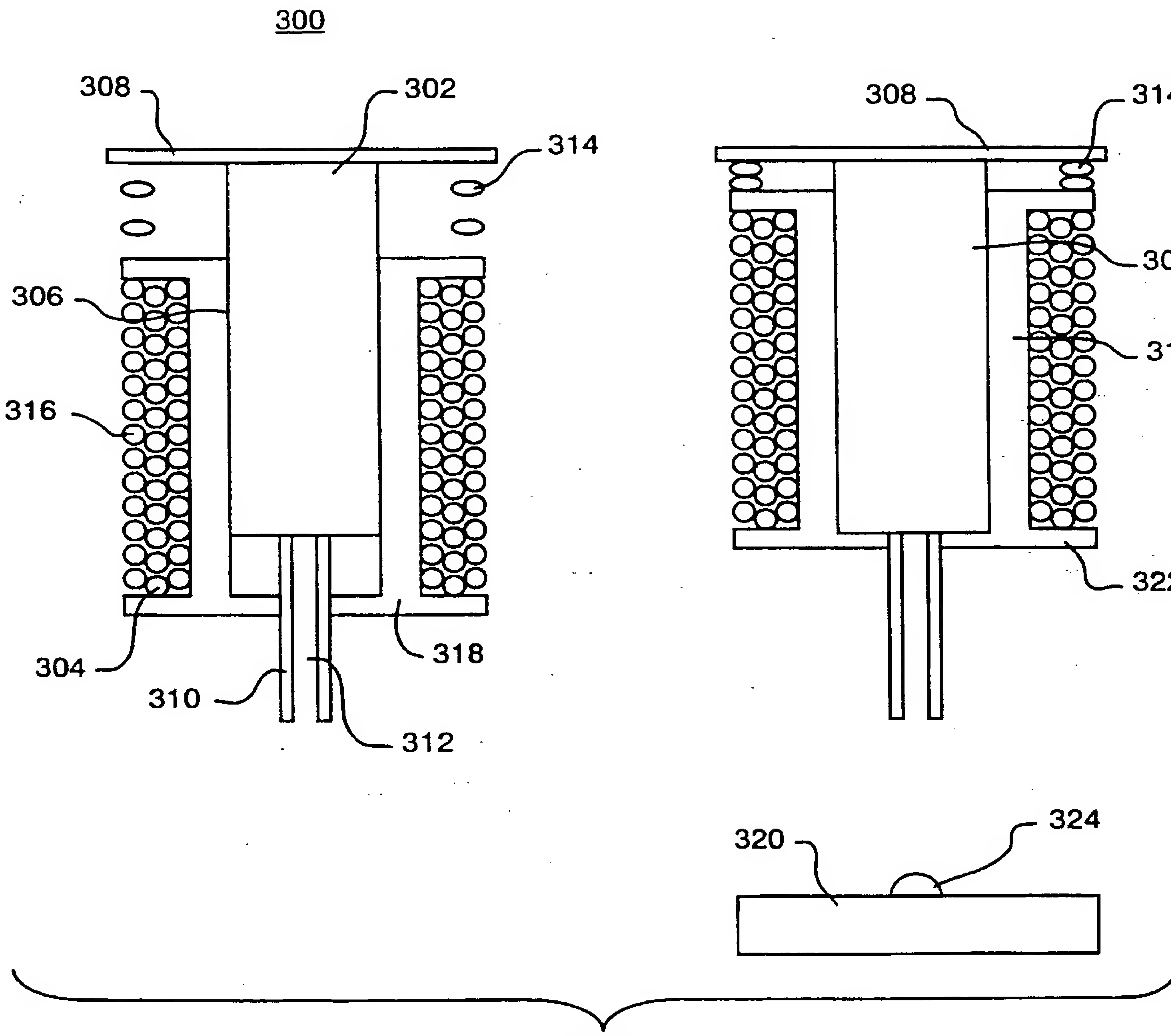
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FIG. 3

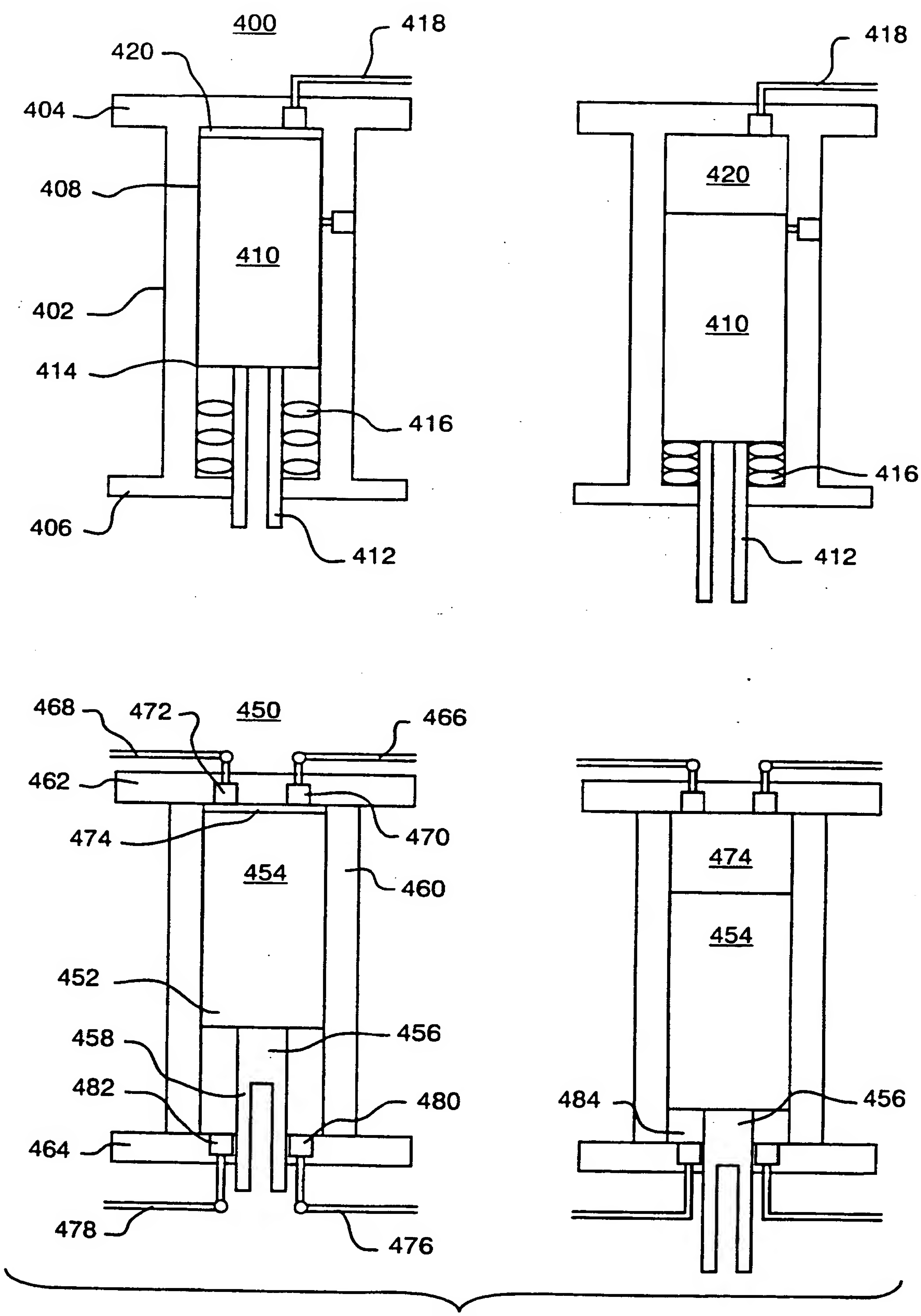
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FIG. 4

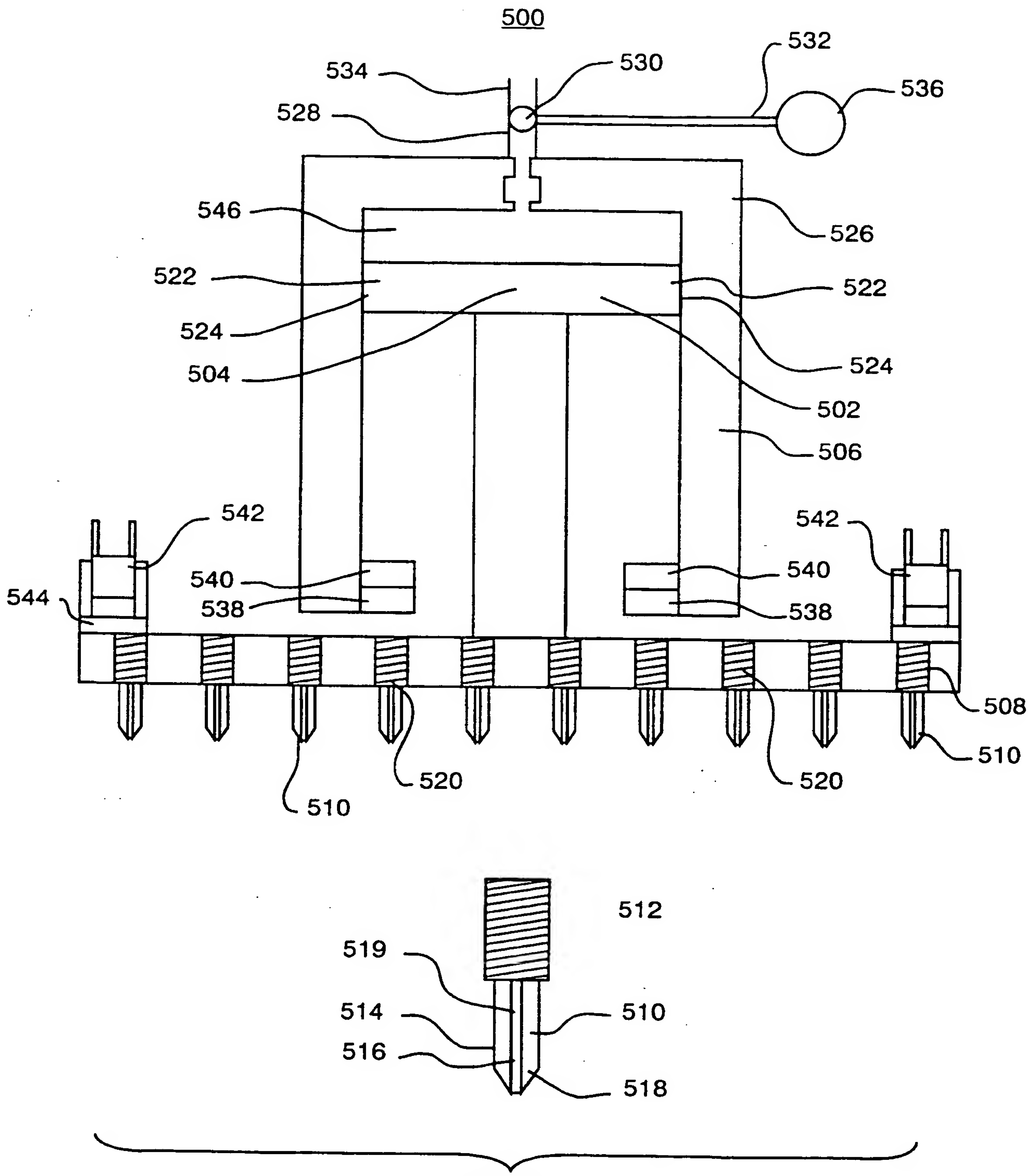
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FIG. 5

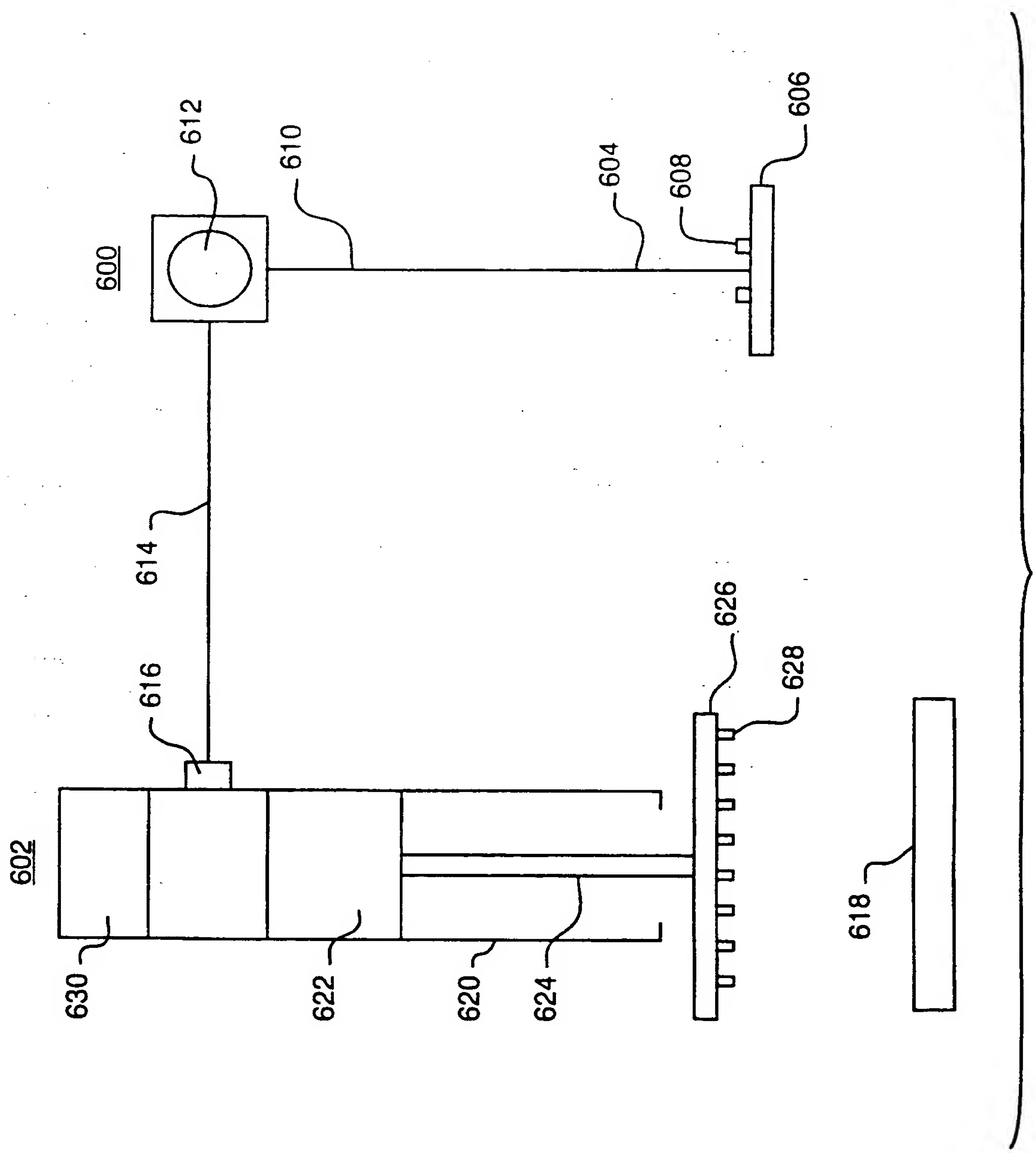
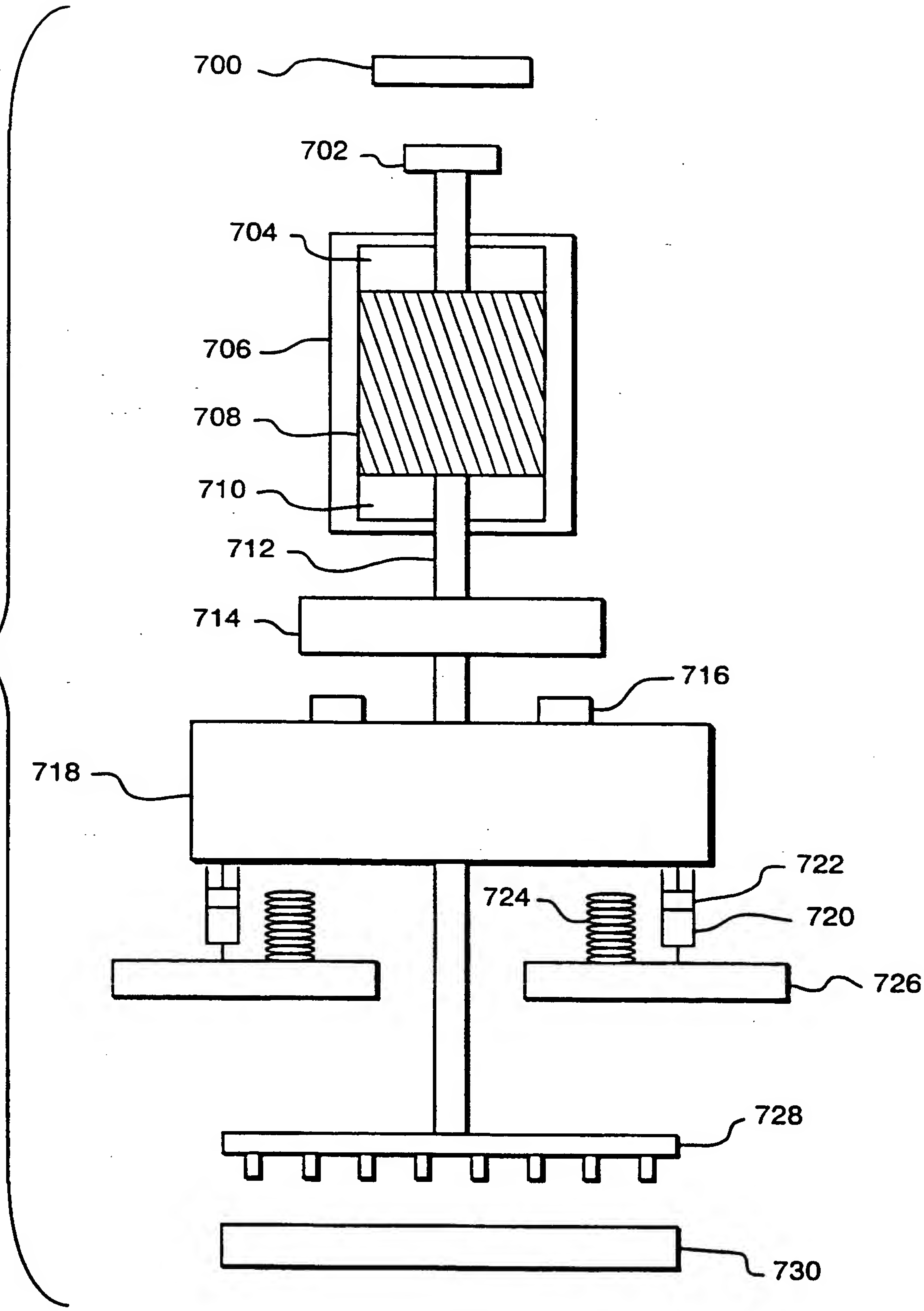



FIG. 6

FIG. 7



# INTERNATIONAL SEARCH REPORT

Inte. .ional Application No
PCT/US 00/25117

**A. CLASSIFICATION OF SUBJECT MATTER**
IPC 7 B01L3/02 //B01J19/00

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01L B01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 99 31468 A (FEYGIN ILYA ;PHARMACOPEIA INC (US)) 24 June 1999 (1999-06-24)<br>page 1, line 7 -page 1, line 20<br>page 2, line 25 -page 3, line 11<br>page 4, line 9 -page 5, line 18 | 1,3-7, 9-12,15, 16 |
| Y | page 6, line 6 -page 9, line 8<br>figures 1-8<br>--- | 2,8,13, 14,17 |
| P,Y | EP 0 955 084 A (CORNING INC)<br>10 November 1999 (1999-11-10)<br>column 1, paragraph 1<br>column 3, paragraph 1<br>column 10, line 9 -column 11, line 6<br>column 15, line 55 -column 16, line 8<br>column 17, line 8 -column 17, line 22<br>figures 1-4,16-18<br>--- | 2,14 |
| | -/-- | |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 29 November 2000 | 07/12/2000 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL – 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Koch, A |

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | WO 99 34214 A (TABONE JOHN C ;GARRISON LORI K (US); RAPIGENE INC (US); NESS JEFFR) 8 July 1999 (1999-07-08)<br>page 10, line 7 -page 12, line 22<br>page 14, paragraph 1<br>figures 1-6<br>--- | 13 |
| Y | EP 0 388 144 A (IRICA INSTR INC ;TAKARA SHUZO CO (JP))<br>19 September 1990 (1990-09-19)<br>column 3, line 20 -column 4, line 13<br>column 4, line 38 -column 5, line 1<br>column 9, line 46 -column 10, line 27<br>figure 6<br>--- | 8,17 |
| A | GB 2 112 664 A (SMITH KENDALL O)<br>27 July 1983 (1983-07-27)<br>page 1, line 74 -page 1, line 119<br>page 1, line 126 -page 2, line 49<br>page 2, line 126 -page 3, line 15<br>page 4, line 8 -page 4, line 56<br>figures 3-7<br>----- | 1,9,10,<br>16 |

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# INTERNATIONAL SEARCH REPORT

**Information on patent family members**

Inte. ɪonal Application No
PCT/US 00/25117

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 9931468 A | 24-06-1999 | US 5957167 A | 28-09-1999 |
| | | AU 1923299 A | 05-07-1999 |
| | | US 6116297 A | 12-09-2000 |
| EP 0955084 A | 10-11-1999 | AU 3552499 A | 16-11-1999 |
| | | AU 3760499 A | 16-11-1999 |
| | | WO 9955460 A | 04-11-1999 |
| | | WO 9955461 A | 04-11-1999 |
| WO 9934214 A | 08-07-1999 | AU 2098999 A | 19-07-1999 |
| | | BR 9814604 A | 17-10-2000 |
| | | EP 1040352 A | 04-10-2000 |
| EP 0388144 A | 19-09-1990 | JP 2242162 A | 26-09-1990 |
| | | US 5089230 A | 18-02-1992 |
| GB 2112664 A | 27-07-1983 | NONE | |